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14. ABSTRACT

Rehabilitation following traumatic brain injury is not well understood and has relied primarily on findings from studies conducted in stroke. We have demonstrated that following CCI (a rodent model of contusion TBI), behavioral function was most enhanced by combining 3 types of forelimb rehabilitation: tray reaching, exercise, and forelimb constraint. This enhancement was seen in tests of Forelimb Reaching but not Forelimb Coordination. Combining all three rehabilitation therapies also enhanced performance on rehabilitation tasks. CCI results in a drastic loss of movement representations in the motor cortex, an effect far more severe than expected based on stroke models. Despite this, the motor cortex near the contusion maintains the capacity for motor map plasticity.

15. SUBJECT TERMS

Controlled Cortical Impact, Reach Training, Exercise, Constraint Induced Therapy, Motor Mapping, Neuroplasticity

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Research Technical Report - Neural Plasticity and Neurorehabilitation Following Traumatic Brain Injury
Dorothy Kozlowski Ph.D. & Theresa Jones, Ph.D.
9/2008 – 10/2009

INTRODUCTION: Individuals with traumatic brain injury (TBI) rarely have the benefit of pharmacological treatments that provide neuroprotection. Instead, they may receive multiple treatments aimed at managing brain injury followed by rehabilitation. While much TBI research is focused on neuroprotection strategies, there remain many individuals who can be expected to either miss the window of opportunity, or to benefit incompletely from early neuroprotective treatment. The focus of this proposal is on treatment strategies for individuals who have passed the acute stage of TBI and rely on rehabilitation. The effects of rehabilitation on the brain have been extensively studied in animal models of stroke and have significantly influenced rehabilitation of stroke patients (for review see (T. A. Jones et al., 2009). These findings and practices are being applied to TBI, however, animal studies examining the effectiveness of rehabilitation and its effect on TBI-induced cellular sequelae and neuroplasticity are lacking. The innovation of the proposed research is that it is the first to examine different types of rehabilitation strategies focused on upper limb function following an animal model of TBI: the controlled cortical impact (CCI). The studies assess the therapeutic utility of these rehabilitative strategies, and examine their impact on the injured brain and on compensatory neuroplasticity.

BODY:

Experiment 1 – Does motor rehabilitative training improve function and promote restorative neural plasticity in remaining brain tissue? Is it improved by adjunctive behavioral therapies?

Conducted at DePaul University, Lab of Dorothy Kozlowski Ph.D.

Rationale: Our labs have demonstrated that compensatory neural plasticity may not occur following TBI. Given this, we believe that forelimb rehabilitation will need to be more rigorous following TBI than what has been shown to be effective following stroke. Therefore, we will employ combinations of rehabilitative training and examine their effects on behavioral outcome and neural plasticity.

Animals: Adult male Hooded Long Evans rats were be placed in the groups. The n's provided are the numbers of animals that are currently completed or underway. (the goal is to have an n=10 in all CCI groups and an n=6 ini all sham groups)

Group:	**n=**
CCI + reach training	9
CCI + reach training + exercise	6
CCI + reach training + exercise + forelimb constraint	3
CCI + forelimb constraint only	0
CCI + yoked control	7
Sham + reach training	5
Sham + reach training + exercise	5
Sham + reach training + exercise + forelimb constraint	3
Sham + forelimb constraint only	0
Sham + yoked control	6

Surgery: Rats received a unilateral CCI over the forelimb representation in the sensorimotor cortex(FL-SMC or Sham surgery (J. E. Minnich et al., in press)

Rehabilitation regime: *Reach training* - animals are trained on a forelimb reaching task prior to the CCI to determine a baseline and preferred forelimb. The rehabilitative reach training of the impaired forelimb (on a tray reaching task) starts on day 3 and is administered once a day until the end of the study (J. E. Hsu and T. A. Jones, 2006). *Reach training + exercise* – reach training occurs as above. In addition, starting at 14 days post-CCI, rats have voluntary access to a running wheel for 6 h/day, 3h each in the light and dark cycle and running distance is measured daily. *Reach training + exercise + forelimb constraint therapy*– reach

training and exercise occurs as above. *Forelimb constraint therapy*- on post-CCI Day 10 rats are placed into limb restricting vests. They are briefly anesthetized with isoflurane so that vests can be customized. The vests are worn continuously for 10 days. Animals in the "CCI+forelimb constraint only" group wear the vests but do not receive reach training or exercise. As a yoked control, non-rehabilitated animals are placed in the reaching chamber and receive pellets on the floor at the same rate that reaching rat retrieves them in the tray. Non-exercised rats receive access to a locked running wheel during exercise periods. Furthermore, animals not receiving forelimb constraint are anesthetized and fitted with control (2-holed) vests during the forelimb constraint period. All vests will be removed on Day 20 prior to final outcome measures.

Behavioral analysis: Forelimb function (of both forelimbs) is analyzed pre-CCI and on days 3, 7, 14, 21, & 28 post-CCI using the forelimb reaching test (single pellet), footfault, vermicelli and limb use tests (J. E. Hsu and T. A. Jones, 2006; J. E. Minnich et al., in press)

Histology: Animals are sacrificed on day 42 post-CCI using intracardiac perfusion. Brains are harvested and sent to Dr. Theresa Jones. Initially we proposed sacrificing on day 30, however preliminary analysis indicated that a slight behavioral effect was starting to be seen on day 30 and that extending the testing period out to day 42 would be beneficial. This change was submitted to ACURO and approved. Therefore, the amount of time spent working with the animals increased from 30 to 42 days.

Preliminary Results:

Unlike what is seen following stroke (T. A. Jones et al., 1999; J. E. Hsu and T. A. Jones, 2006; M. A. Maldonado et al., 2008), rehabilitation reach training alone was not sufficient to enhance behavioral function following CCI. In a sensitve motor assessment task, the single pellet test, the group that benefited the most was the one that received all three rehabilitative reaching paradigms (See Figure 1). Interestingly, combining reach training with exercise may also provide an additive benefit (this was not seen following stroke (M. A. Maldonado et al., 2008). This behavioral enhancement however is seen in the single pellet test, but not the foot fault test. We are currently still analyzing videotapes of limb use and vermicelli and cannot comment on the benefits of rehabilitation on those tasks at this time.

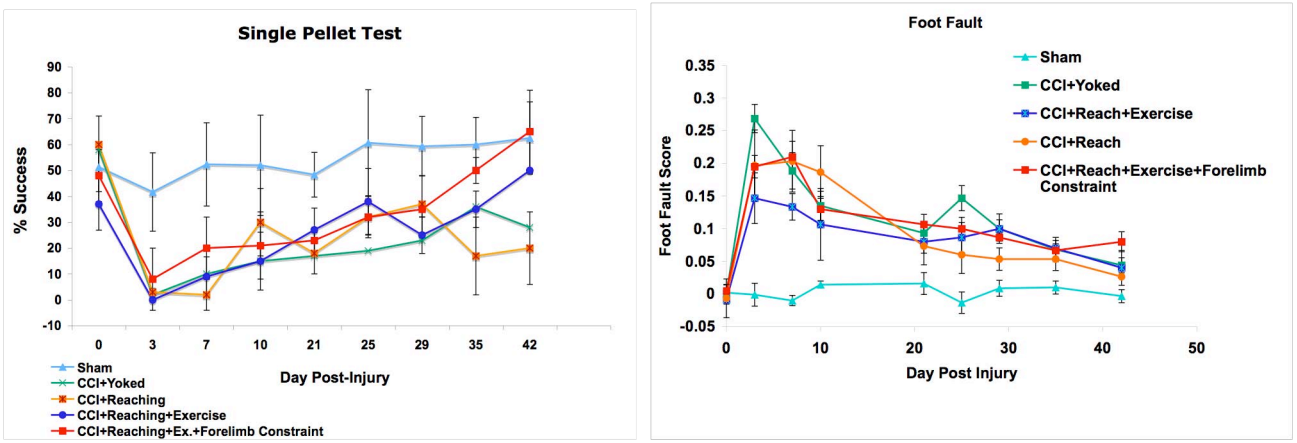


Figure 1. – Combining all 3 rehabilitation tasks enhances success rate on the single pellet test but does not affect forelimb coordination as measured by the foot fault.

Combining all three rehabilitation tasks also seemed to enhance performance on the rehabilitation tasks themselves. Analysis of the time it takes to complete the tray reaching rehabilitation task demonstrated that animals that received all 3 rehab tasks completed the tray reaching faster, and also seemed to run further in the exercise wheel. See Figures below – next page.

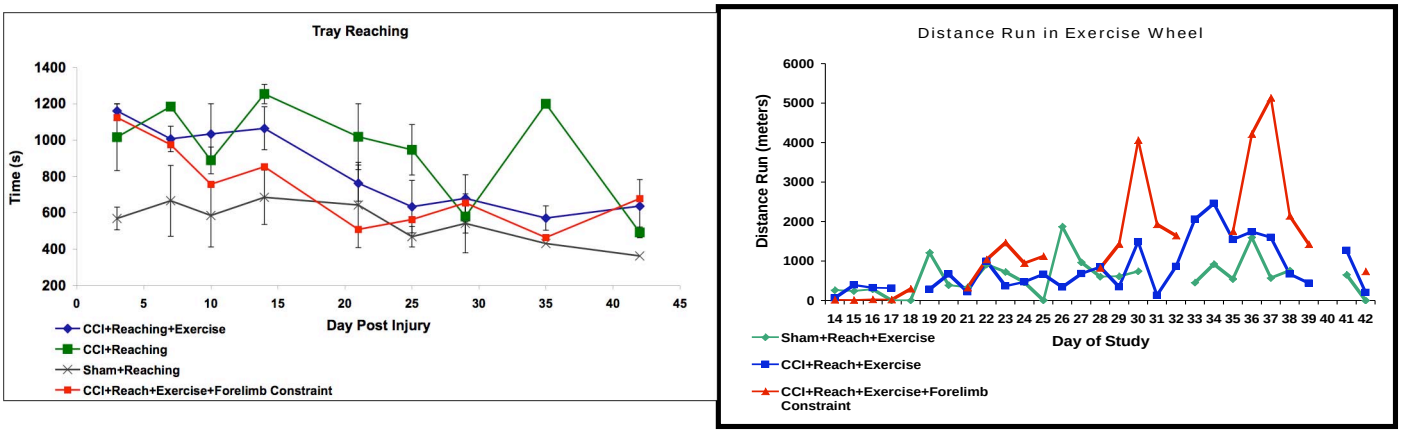


Figure 2. Combining all 3 rehabilitation tasks enhances the performance of the injured animals on the tray reaching tasks (left) and increases the distance they run in the exercise wheel (right).

Histology, cont.: The brains will be sliced serially and coronaly into sets and immunohistochemically analyzed for the following: contusion size estimated as volume of remaining tissue in Nissl stained sections, MAP2 (dendritic structural plasticity), synaptophysin, sinophilin (pre- and post-synaptic markers, respectively), GAP-43 (axonal growth-related protein), NF200 (axon marker). Quantitative analysis will rely on stereological measures or optical densitometry, as appropriate, given staining patterns. Sample locations will be the remaining sensorimotor cortex around the injury, in the contralateral homotopic cortex and subcortical structures, including the striatum and thalamus (Hsu & Jones, 2006).

Preliminary Histology Results: The animals listed above have been sacrificed, brains harvested and sent to the lab of Theresa Jones for slicing and staining. To date, the brains have been sliced and one set stained for Nissl. Using the Nissl stained sections, Dorothy Kozlowski's lab has analyzed the size of the contusions. Previous studies have shown that if animals receive too strenuous of a rehabilitation regime too early, it may result in an expansion of the size of the injury. We have demonstrated that there are no significant differences in contusion size at this time. See Figure 3 below.

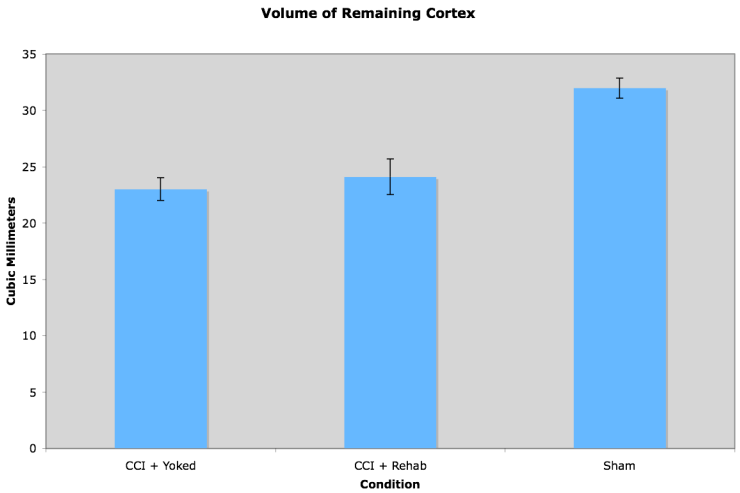


Figure 3. Volume of remaining cortex is significantly decreased in both CCI groups compared to Sham. However the animals receiving rehabilitation do not have a larger contusion size than CCI+yoked animals.

The remaining sets of brain sections are going to be stained with markers of plasticity using immunohistochemistry. Because Immunohistochemical staining can be greatly altered due to batch effects, we are waiting until we get the majority of brains from the rats before Immunohistochemical staining will take place.

Experiment 2 – Does motor rehabilitation promote synaptic structural and functional plasticity in remaining motor cortex following TBI?

Rationale: After stroke, functional improvements resulting from motor rehabilitation focused on an impaired forelimb has been found to induce functional reorganization and synaptic plasticity in remaining motor cortex. This experiment will determine whether a rehabilitation regime shown to be most effective in improving function in Exp. 1 also results in parallel plasticity of maps and synapses. In addition to validating and extending the results of Exp.1, this study is important for revealing potential mechanisms of functional recovery that might be manipulated or facilitated to further improve function.

Animals: Adult male hooded Long Evans rats will be placed in the following groups. Power Analysis was conducted as in Exp. 1. An "n" of ten animals per group was conservatively estimated.

Group:	n=	
CCI+Reach Training (+ adjunctive therapy depending on Exp.1)	10	
CCI+Control	10	
Sham+Reach Training (+ adjunctive therapy depending on Exp.1)	10	
Sham+Control	10	TOTAL =40

Motor Mapping and Electron Microscopy (EM): Synaptic structural and functional plasticity of remaining motor cortex will be assayed using stereological methods for quantitative EM and intracortical microstimulation (ICMS) mapping, respectively, within the same animals. CCI, reach training and behavioral assays will be as in Experiment 1. All digital videotapes of behavioral measures will be sent to the Kozlowski laboratory for analysis. Dr. Kozlowski will travel to UT-Austin to conduct the CCI's. On day 30 post CCI, the caudal and rostral forelimb areas and surrounding regions of both hemispheres will be mapped using standard rodent ICMS techniques under ketamine-xylazine anesthesia, a procedure established in collaboration with Jeffrey Kleim (unpaid consultant). In this method, the organization of motor cortical movement representations are revealed in detail by stimulating primary motor cortex in a systematic (grid like) manner. In this initial experiment, mapping will not be conducted multiple times (because repetitive ICMS may impact the CCI or the EM), but it is expected that this study will set the stage for future within-animal analyses of the time course of map changes. Immediately after mapping, identified cortical forelimb representation regions of either hemisphere will be processed for EM. The density and ultrastructural characteristics of synapses in the motor cortex will be assayed using well-established quantitative approaches.

Preliminary Results:

To date, the Jones lab has obtained the Benchmark Impactor and with the help of Dr. Dorothy Kozlowski, they have contused a number of animals to verify that their device is producing comparable injuries (CCIs) using the same parameters as are used in the Kozlowski Lab.

Dr. DeAnna Adkins has become very proficient in the CCI model with the guidance of Dr. Kozlowski and has begun performing CCIs and mapping animals for the CCI+ Control Group. Experiment 2 relies on knowing the results of Experiment 1 and we just recently feel comfortable with the fact that the group that seems to be most beneficial to perform motor mapping and EM on will be the group that receives CCI+Reach Training+ Exercise+ Forelimb Constraint. We are preparing for those studies now.

Dr. Adkins and Dr. Jones have begun another study in which they are performing cortical stimulation of the FL-SMC coupled with reaching to examine whether cortical stimulation can be beneficial as an adjunct to

physical rehabilitationfollowing TBI. Although this study is not funded by the DOD, the preliminary results of these experiments relate directly to Specific Aim 2 of this grant and will be mentioned here.

Rats received a CCI over the FL-SMC and an electrode was implanted on dura over the peri-injury Fl-SMC. On day 10 post-injury rats began receiving rehabilitative tray reaching and cortical stimulation (CS) (at 50% of movement threshold) daily for 9 weeks. During that time behavioral testing using single pellet test was conducted. At 9 weeks post-injury the motor cortex was mapped using ICMS in rats that received CCI+Reaching+CS and CCI+Reaching only.

Preliminary behavioral results demonstrate that rats that received both CS+Reaching performed better on the single pellet task than those that received reaching alone (See Figure 4 below).

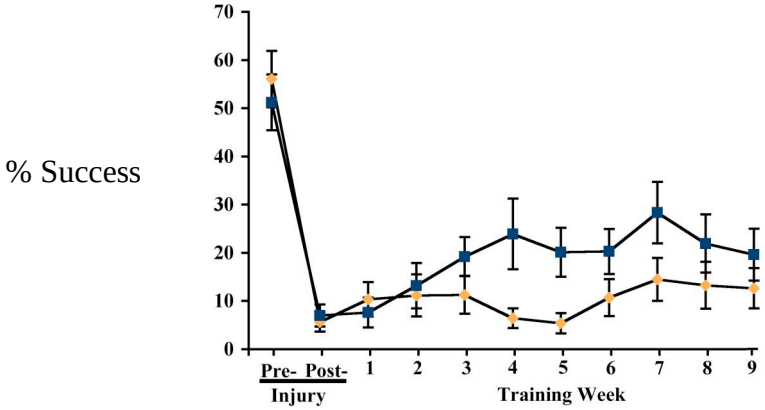
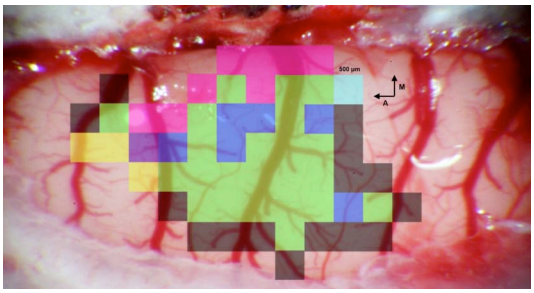


Figure 4. Percent Success at the Single Pellet Test shows that animals that receive CS+Reaching (Blue) were more successful than those receiving reaching alone (yellow).

Preliminary results of the Motor Mapping Studies (ICMS) show that in only 2 out of 6 animals tested, were forelimb movements evoked at the threshold criterion of ≤ 100 μA in the injured cortex. Examples of two different post-TBI motor cortical movement representation maps after 9 weeks of tray reaching are shown below (right). This suggests that cortical reorganization is significantly limited following TBI.



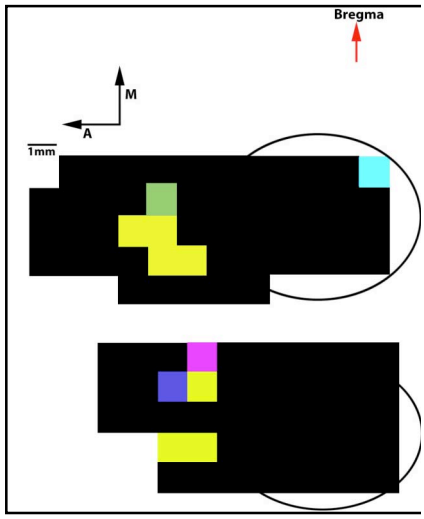


Fig. 5 - Example of ICMS derived movement representation maps from an intact motor cortex (Left) and following CCI – (Right). Colored boxes correspond to different movements:

Black = no response
Pink = whisker
Green = wrist/digit
Blue = elbow
Light blue = hind limb
Yellow = jaw/neck

KEY RESEARCH ACCOMPLISHMENTS:

- Animals are being trained, injured, rehabbed and tested in Dr. Kozlowski's lab
- Brains are being harvested and sent to Dr. Jones' lab
- Dr. Jones' lab is slicing the brains, staining one set with Nissl, saving the remaining sets for Immunohistochemical staining.
- Dr. Kozlowski's lab is analyzing contusion size.
- Dr. Kozlowski's lab is analyzing videotapes of behavioral tests.
- Dr. Jones lab has established the CCI model in their lab with the assistance of Dr. Kozlowski.
- The Jones' lab has shown that CCI's conducted at UT-Austin are comparable to those conducted at DePaul University
- They have performed ICMS mapping on 6 injured animals and are ready to begin mapping studies on animals that receive all 3 rehabilitation treatments.

REPORTABLE OUTCOMES:

Poster/Oral Presentations:

Ferguson, L., Adkins, D.L., McDonough, K., Stamschror, J., Colella, M., Jones, T.A., & Kozlowski, D.A. Cortical electrical stimulation of the motor cortex, exercise, and motor rehabilitative training after a controlled cortical impact to the forelimb sensorimotor cortex: Differential effects on skilled motor function. *Society for Neuroscience Meeting, 2009.*

Kozlowski D.A., & Jones, T.A. Neurorehabilitation and Neuroplasticity Following Traumatic Brain Injury. *Military Health Research Forum 2009. (poster and oral presentation)*

Adkins DL, Tennant KA, Donlan N, Jones TJ, Kozlowski D (2009) Cortical electrical stimulation of the motor cortex after a controlled cortical impact to the forelimb sensorimotor cortex improves skilled motor function. *National Neurotrauma Society Abstract #226. Peer Reviewed*

CONCLUSIONS:

- **Following CCI, behavioral function was most enhanced by combining tray reaching, exercise, and forelimb constraint. This enhancement was seen in tests of Forelimb Reaching but not Forelimb Coordination.**

- **Combining all three rehabilitation therapies enhanced performance on rehabilitation tasks.**

- **Rehabilitation does not increase the size of the contusion.**

- **CCI results in a drastic loss of movement representations in the motor cortex, an effect far more severe than expected based on stroke models.**

- **Despite this, the motor cortex near the contusion maintains the capacity for motor map plasticity.**

This study demonstrates that neural plasticity may be limited following TBI and that the rehabilitation protocol required to produce behavioral enhancements is more extensive than that seen in a similarly sized lesion due to stroke. However, it also indicates that the capacity for functionally beneficial reorganization of motor cortex is possible.

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Minnich JE, Mann SL, Stock M, Stolzenbach KA, Mortell BM, Soderstrom KE, Bohn MC, Kozlowski DA (in press) Glial cell line-derived neurotrophic factor (GDNF) gene delivery protects cortical neurons from dying following a traumatic brain injury. Restor Neurol Neurosci.

APPENDIX:

None.